CYTOGENETIC STUDIES OF AFRICAN AWNED SQUILLS (DIPCADI SPECIES) IN NIGERIA.

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DEDICATION

This work is dedicated to my parents, MR. & MRS. ADELANWA, and to my uncle, MR. OLUSEGUN AKINWUMI, the starter and motivator of my education career.

ABSTRACT

Nigerian representatives of the African Awned Squills (Dipcadi Medic) were sampled from natural populations examined during ft'eld survey and collection trips. These representatives were grown in botanical nurseries in both University of Ilorin and Ahmadu Bello University, Zaria. Three taxa were recognised. Chromosome counts and karyotype study were conducted on the three taxa while meiotic chromosome study was carried out on two of the three taxa. The genus Dipcadi was found to be represented by two ploidy levels: diploid, 2n = 12 and tetraploid, 2n = 24. Dipcadi tacazzeanum and Ih filamentosa were diploids while D^ longifoluim is a tetraploid. The two diploids were karyotypically diferent. Meiosis was found to be regular in D. tacazzeanum, with six bivalents, and D^ longifolium with twelve bivalents. Experimental evidence indicated that all the Nigerian representatives are of hybrid origin.

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I owe a special thanks and gratitude to my Mum, MRS. OMOLOLA ADELANWA for being my constant companion and unfailing source of inspiration.

Finally, and above all, I give honour, glory and praises to Almighty God for His divine mercy and grace over me.

CERTIFICATION

This is to certify that the work reported in this thesis was done by the candidate, Mr. M.A. Adelanwa under the supervision of Prcfessor S.O. Oyewole in the Department of Biological Sciences, University of Ilorin.

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APPROVAL SHEET

We certify that this work was carried out by Mr. M.A. Adelanwa and approved as meeting the requirement for the award of M.SC degree in Botany in the Department of Biological Sciences, University of Ilorin, Ilorin, Nigeria.

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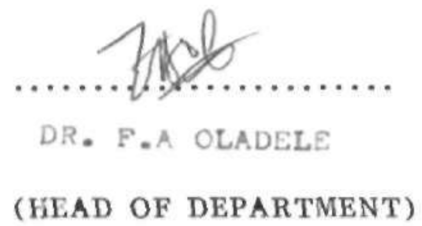

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EXTERNAL EXAMINER DATE

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CHAPTER ONE

INTRODUCTION

Liliaceae is one of the largest groups of the angiosperm Families with representatives all over the world and in all conceivable ecological niches. The phylogeny is viewed conservatively as one of the less advanced monocotyledonous families. The evolution of the family has been subject to speculations by evolutionists and systematists. Recent works, however, have shown that the family is evolutionarily active and dynamic. Little research interest has been expended on the family apparently because of the growth habit of most members. They are mostly known as potted ornamentals in homes and horticultural garden plants. Some genera such as Urginea Stein and Dipcadi medic are known to possess potent alkaloids that have poisoning effect on rats and insect pests most especially when dried, ground into powder and mixed with animal feeds. The drug colchicine was first discovered from Colchicum L.

The family is subdivided into 28 tribes each of which consists of related genera. Only six of these are found in Tropical West Africa. These are Asparageae, Aloineae, Hemerocallideae, Asphodeleae, Colchiceae, and Scilleae (Baker, 1752). The tribe Scilleae is a genus of lilies whose members are generally referred to as African awned squills (Morton 1961). They grow mostly in dark humus soil on extended rocky outcrops. Based on external morphological features. Morton,

(1961) recognised D. filamentosa while Hepper (1968), in the latest revision of the family, recognised two species in the genus, namely, D. longifolium and D. tacazzeanum.

In Hepper's revision, Morton's D. filamentosa was sunk into D. tacazzeanum. Jones and Smith (1967) referred to another species D. gracillium which is not in Tropical West Africa. Oyewole (personal communication), in his extensive study, identified three distinct taxa which approximate to original descriptions of D. filamentosa, D. tacazzeanum and D. longifolium. Gledhill (by personal note on herbarium materials) noted great variations in the morphology of the representatives of the genus and advocated for its revision. With apparent similarities in the morphology of the members of the genus, the taxonomy and nomenclature of this group of plants are in a confused state. Although, efforts were made some years ago to revise the taxonomy of the family (Hepper 1968), these have produced very limited results as admitted by Hepper himself, and very little information has been added to the existing information on the genus. With all the seemingly conflicting taxonomic conclusions on this genus with so few a number of representatives in Tropical West Africa, it becomes therefore evident that more information is needed to resolve the genus into appropriate taxa. In the light of this, accumulation of data from cytogenetics has been found to be most desirable for this purpose.

Cytogenetics is a hybrid of cytology and genetics(Swanson, 1965) It has played an immense role not only in species delimitation but also in determining relationship and the course of evolution among related taxa. Cytology is undertaken with the hope of establishing the physical basis of heredity so as to throw more light on the phylogeny and inter-relationship of multitudinous forms of living organisms through studying the structure, behaviour, growth, reproduction of cells and exploring the properties of the cytoplasm (Darlington, 1928; Sirks, 1956; and Gardner, 1965). Cytological investigation presents one of the most reliable data in resolving taxonomic problems. This has been amply demonstrated in the classification of many plant taxa such as Trillium (Darlington and Shaw 1959; Serota and Smith 1976). Oyewole, (1972) employed the data obtained from cytological investigationary in formulating relationship and evolutionary trends among the species of Albuca in West Africa. Oyewole, (1975) also employed cytological studies in resolving the taxonomy of Urginea altisima complex in West Africa. The same studies have equally been utilized to elucidate the taxonomy of various angiosperm taxa such as Luzula campestris (Buchanan, 1958), Dubantia and Railliardia (carr, 1978). Naik, (1976) applied cytogenetic data to postulate the probable evolution of the Indian species of the genus Chlorophytum. This same approach was employed by Adeyemi, (1981) in his biosystematic studies

of the genus Chlorophytum in Nigeria. Cytogenetic studies could also highlight the hidden cause of some externalized abnormal behaviours. A good example is seen in the work of Oyewole (1984) where the knowledge of cytogenetics was employed in detecting the cause of sexual sterility in Drimiopsis barteri.

Genetics on the other hand, deals with heredity which seeks to explain the similarities and differences that exist between parent and their offsprings (Mc Graw-Hill, 1977) and between closely related species, genera and even tribes. When phylogenetic consideration enters into taxonomy, the reasons for similarities between present day organisms become more understandable as being the consequence of common descent and common genetic make-up (Heywood, 1976). In order to establish a comprehensive and convincing cytogenetical relationship between two morphologically related organisms at any taxonomic level, genetical investigations need to be carried out. This is important mainly because the phenotypic characters are manifested by the genes located in the chromosomes, the plasmagenes or cytogenes (i.e functional parts of chromosomes) found inthe cytoplasm which can multiply by themselves completely and independently, (Wright, 1941; Sirk, 1956; and Webster 1966) in interplay with environmental factors which play a modifying role. Karyotype is the appearance of the somatic chromosomes at mitotic metaphase. This involves the studies of

some taxonomic characters which include chromosome counts, chromosome morphology, chromosome length, position of the centromere, presence or absence of secondary constriction with satellites and presence and distribution of heterochromatin. The usefulness of karyotype data in complemeting other taxomomic information from other areas of study has been emphasized in a considerable member of works (see Jones and Smith, 1967; Sharma et al, 1969; Barber, 1970). Karyotype analysis has been a useful tool in the classification of many plants such as Trillium (Darlington and Shaw 1959; Serota and Smith, 1967). Variation in size and form of chromosomes between genera and related species are of importance in their re solution into the appropriate taxa (Sato 1962).

Unlike in the past, a substantial work has been done on members of the family liliaceae. But the same cannot be said of the cytogenetic studies in the genus Dipcadi. Apart from the extensive studies carried out by Oyewole (personal communication), where he identified three distinct taxa, there is still paucity of information, thereby still leaving unresolved the problem of the taxonomic status of the identified taxa of the genus. This present study therefore aims at carrying out cytogenetic studies on members of Dipcadi with a view to:

1. Resolving them into appropriate taxa
2. Elucidate the taxonomy of the genus and
3. Be able to make a pronouncement on the genetic relationship of the members as well the geneology of the individual members.

CHAPTER TWO

MATERIALS AND METHODS

Trips were taken to different areas both within and outside Kwara state between December 1995 and December 1996 where many live bulbs were collected from wild population at different localities. The trips were undertaken at different times in both dry and rainy seasons and collected population samples were brought into the nursery for cultivation. Specimens were brought to the herbarium for proper identification. The areas or localities visited include:

- An area about two hundred meters away from the main road, behind Kwara Hotel complex, where population of bulbs were found growing in humus soil near the wall of St. Anthony's Secondary school, Ilorin. Many bulbs were collected at this location.
- An extensive, flat rocky outcrop located in the North Eastern part of the University of Ilorin main campus was also visited where several bulbs were collected.
- Collection of bulbs was also made during a trip to Ijagbo via Offa where materials were found growing in dark humus soil on low rocky extensive outcrop about five hundred meters behind Jofans Construction limited, along Offa, Ilorin road.

Fig. 1 Map of Nigeria showing routes during field trips and areas of major sampling.

- Areas of collection of D. longifolium
- Areas of collection of D. tacazzeanum
- Areas of collection of D. filamentosa

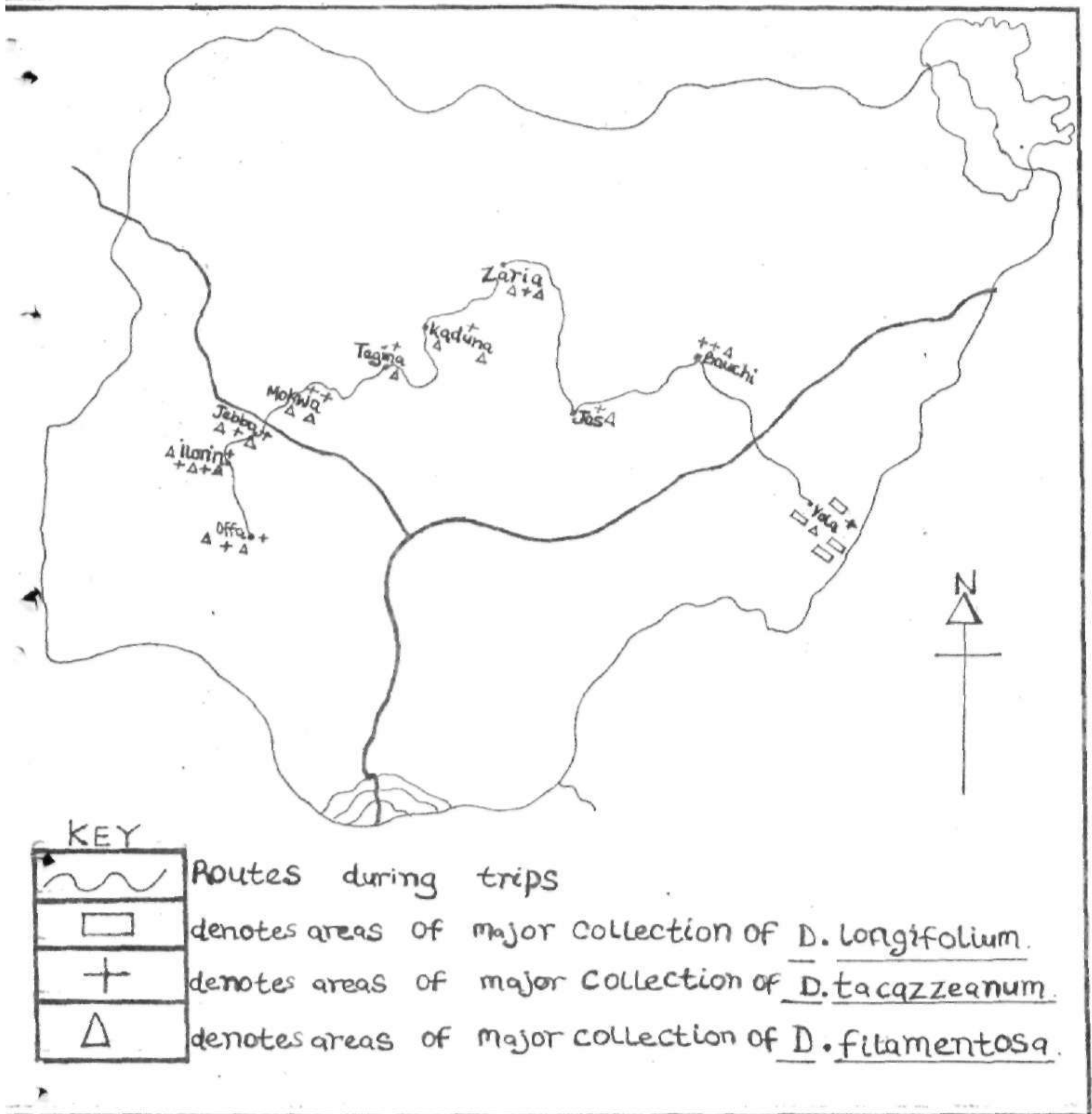


Fig 1

- Genetically viable bulbs were also collected during a long trip to Yola where they were found growing on rock crevices along Mubi road.
- Kufena rocky outcrop in Wusasa - Zaria and the Botanical garden of Ahmadu Bello University, Zaria were also visited where viable bulbs were collected.

Collections after proper identification included bulbs of Urginea, Albuca and Drimiopsis.

(i) MORPHOLOGICAL DIFFERENTIATION OF COLLECTED TAXA

After taken a critical observation on the morphology of the collected bulbs, three distinct taxa were eventually recognised as opposed to Hepper's recognition of two species. It was confirmed that Dipcadi longifolium and D. tacazzeanum show great morphological variations especially with different soil types; both are more luxuriant in dark humus than in gravely or sandy soil. D. filamentosa on the other hand was found usually associated with drier or shallower or more brownish soil and is smaller in all respects, most especially in bulb size, root thickness, leaf length and width, reproductive shoot height and thickness, size of

inflorescence and fruit size. Apart from these, its leaves are generally twisted and more pointed at the apex.

Dipcadi tacazzeanum and *D. longifolium* are easily differentiated from each other by the pinkish leaf base and reproductive shoot, stoutness of the reproductive shoot, and size of the fruit.

(ii) VEGETATIVE MORPHOLOGY OF COLLECTED TAXA

The bulb is ovoid to spherical with white to cream coloured scales and membranous tunics. The basal stem is discoid, less than 5mm high, and the bulb is various in size. Reproductive shoot is borne among vegetative leaves; the inflorescence is also borne in the distal half of the shoot. Flowers are pedicelled, mauvre in colour with biseriate six segmented perianth, segments connate at the base; the outer reflexed at anthesis. Bract is pyramidal - lanceolate, persistent as in Albuca lin. but are tailed.

D. longifolium

Bulb size vary from one soil type to the other, almost spherical with an inconspicuous basal stem. Leaf size also vary, pink and chanelled at the base, lamina green and straight; reproductive shoot length also vary depending on soil type stouter than in D. filamemtosa and D. tacazzeanum, pinkish in the proxinal part. Inflorescence many flowered and 30 - 100cm long. Bract is about 12mm long. Outer tepal is about 13mm long with tongue up to 2 - 3mm long. Inner tepal 9 - 10mm long; filament is 5 - 6mm long; anthers about 3mm long. Ovary is oblong and 3mm in diameter while the style is about 2.5mm long. Fruit 10 - 15mm long; seeds are black and papery.

<u>D. filamentosa</u>

Bulb is oblong or ovoid and 30 - 50mm in diameter. Leaf up to 20 - 30cm long and 10 - 15mm wide, twisted and light green. Reproductive shoot is variable, 8 - 30cm in height, produced in late march after the first rain in Ilorin. Inflorescence 15 - 18cm long, with few flowers usually not more than eight. Pedicel about 11mm long; bract is about 16mm long. Outer tepal 5 - 6mm long. Filaments are about 4.5mm long while anthers are 3 - 4mm long. Ovary 2 - 3mm in diameter; style about 2.5mm long. Fruit 10 - 14mm long.

<u>D. tacazzeanum</u>

Bulb diameter vary from one soil type to the other, it is ovoid with obvious basal stem. Leaf size also vary, dark green, usually straight. Reproductive shoot length also vary depending on the soil type, produced in late March after the first rain in Ilorin. Inflorescence 15 - 16cm long bearing up to 15 flowers. Pedicel about 11mm long; bract not less than 10mm but up to 17mm long. Outer tepal 8 - 9mm long with tongue 2.5mm long. Inner tepal 5.5 - 6.5mm long. Filament about 4mm long. Anthers 3.5mm long and with slightly expanded base, thick and about 4mm long with divergent base. Ovary 2.5mm in diameter. Style 2mm long. Fruit about 10 - 15cm long.

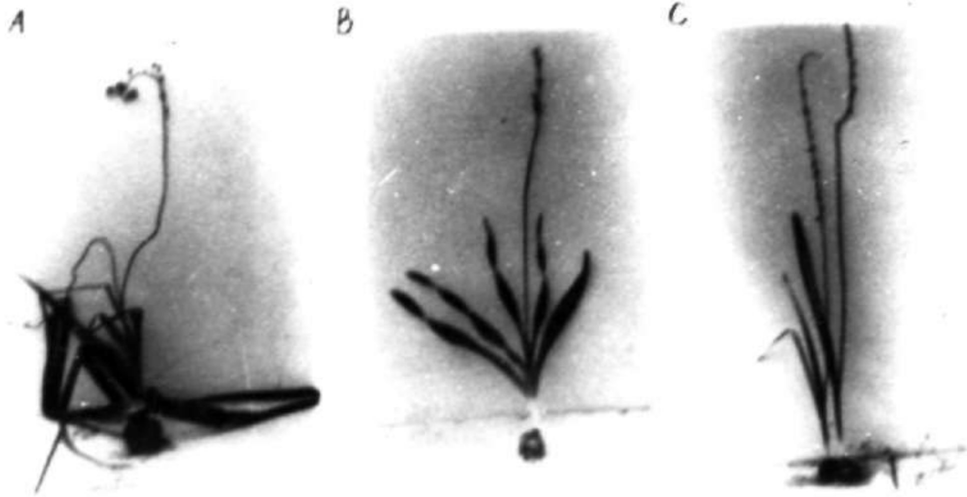
PLATE 1

Vegetative morphology of the three taxa of *Dipcadi*

A - *D. tacazzeanum*

B - *D. filamentosa*

C - *D. longifolium*



× 2

It is not always easy to identify or differentiate the three taxa morphologically except when they are collected together in the field. Observation was made that the aerial part of the plant's size is greatly dependent on the fertility of the soil.

KEY FOR SEPARATING TAXA

1. **Leaf Straight.**

    2. Reproductive shoot and leaf base pink.

    Bulb, vary in size depending on soil type, almost spherical with an inconspicuous basal stem; inflorescence length also vary with soil; bract about 12mm long; outer tepal is about 13mm long; filament is 5 - 5½mm long; anthers about 3mm long; ovary is oblong and 3mm in diameter; style 2½mm long; Fruit 11 - 16mm long .... D. longifolium.

    2. Reproductive shoot and leaf green.

    Bulb, also vary in different soil type, ovoid with obvious basal stem; inflorescence length also vary in different soil; bract not less than 10mm but up to 17mm long; Outer tepal 8 - 9mm long; Inner tepal 5.5 - 6.5mm long; filament with slightly expanded base, thick and

about 4mm long; anthers 3.5mm long; ovary 2.5mm in diameter; style 2mm long; Fruit 10 - 15mm long .... D. tacazzeanum.

1. **Leaf twisted**

Bulb, 30 - 50mm in diameter, oblong or ovoid; inflorescence 15 - 18cm long; bract 16mm long; outer tepal 8mm long with tongue 2 - 3mm long; inner tepal 5 - 6mm long; filaments about 4.5mm long; anthers 3 - 4mm long; ovary 2 - 3mm in diameter, style 2.5mm long; fruit about 10 - 14mm long .... D. filamentosa.

(iii) KARYOTYPE STUDY

The karyotype study was carried out using the root tip squash. This is because the bulbs can easily grow young healthy roots.

(a) Harvesting of Materials

Bulbs were grown in plastic pots filled with sandy loam. The pots were flooded with water so as to allow easy removal of the tender roots without damaging them. Young tender growing roots not longer than 2 - 2.5mm were harvested from

each bulb between 8.00a.m and 9.00 a.m. from time to time. The growing root tips whose cells are known to be in active division between 6.00 a.m and 9.00 a.m. (Oyewole, 1984a) were removed using a pair of fine scissors.

(b) **Pretreatment**

The root tips were then pretreated by placing them in specimen tube containing aqueous solution of paradichlorobenzene (P-DCB). This was kept in a dark, well ventilated cupboard for 60 minutes. After a period of one hour the P - DCB was poured away from the tube and the root tips rinsed thoroughly twice using distilled water. Pretreatment is normally undertaken with a view of obtaining shorter and thicker chromosomes which would facilitate easy counting (Morton 1962). When chromosomes are in this condition, they become more conspicuous and definitive in morphology. Pretreatment also removes undesirable deposits in the tissue which facilitates rapid fixation of material. (La cour 1935).

(c) **Fixation**

Fixation is the process by which a chemical or mixture of chemicals (fixative) added to living materials arrest all physiological activities of the cells and instantly kills all

cellular organelles. The root tips were carefully transfered one by one using a pair of forceps into a specimen tube containing freshly prepared 1:3 (V/V) glacial acetic alcohol (Darlington and LaCour, 1966). They were stored in the refigerator at 4°c until they were needed. Fixation is normally employed to arrest and kill the cells and make them static for cytological study. It should not only increase the visibility of the chromosome structure, but should also clarify the details of chromosome morphology, such as the euchromatic and heterochromatic regions as well as the primary and secondary constrictions.

(d) **Hydrolysis**

When needed for cytological studies, the fixative was poured away while the root tips were properly rinsed in quick changes of distilled water. they were then hydrolysed in 5N HCl for about 8 minutes at normal laboratory temparature (Oyewole, 1971). This is necessary for softening the tissue by dissolving the middle lamella of the cells so that squashing is possible for proper study of the karyotype. Care was adequately taken in the determination of appropriate timing to prevent overhydrolysis which would result to poor staining,

or underhydrolysis which would make squashing difficult or unmanagable.

(e) **Staining and Squashing**

The hydrolysed roots were rinsed in two quick changes of distilled water. The root caps were then teased away using two mounted needles. The meristematic region was gently ripped off unto the surface of a clean glass slide while the older portion was discarded (La-Cour, 1962). Squashing and staining were done simultaneously. Two drops of working solution of acetic orcein (1%) stain was added onto the meristematic region and gently tapped with the round base of the mounted needle. The turbid suspension formed was gently covered with a clean cover slip smeared with a thin film of glycerine albumen. The slide was then warmed on the spirit lamp, and pressed between two folds of filter paper to remove excess stain. This was repeated until a satisfactory spreading of the cells was obtained.

(f) **Chromosome Count and Measurement**

Observation of the chromosome morphology and counting of the number of chromosomes in a complement was done using x100 objective of the Olympus Research Microscope. with the use of calib rated eyepiece graticule, the total chromosome

length (C), the length of the long arm (L) and the length of the short arm (S) of each cromosome were measured. The centromeric value (r) is given as the ratio r = L/S (Levan et al, 1964). Twenty four cells from twelve root tip preparations of each group were examined and average measurements for each species determined.

(g) **Photography and Construction of Idiogram**

Photomicorgraphs of well spread complements at the metaphase stage were taken at x100 objective of the olympus M20 Research microscope. The film used and found appropriate was ORWOPAN (black and white) film. The construction of idiogram was achieved using the haploid chromsome number where chromosomes were measured to show the length of both the long and short arms as well as the relative positon of the centromere. The chromosome lengths were arranged in descending order in the idiogram. The heavily stained regions of the chromosomes were identified as heterochromatic regions according to the procedure of Brown (1949).

(h) **Permanent Slide Preparation**

With the aid of a diamond pencil, three of the corners of the cover slip were marked on the slide. This aided in

locating the exact position of the cover slip at the end of the exercise. The slide was then placed facing downward in a staining tray containing acetic alcohol. After 2 - 5 minutes, the cover slip would fall off. At this stage excess stain from both the slide and the cover slip was removed. Slide and cover slip were removed separately and transfered into another tray half filled with absolute ethanol. The slide and the cover slip were properly placed to maintain the same position as in the acetic alcohol. The cytological materials were then allowed to be adequately dehydrated for two minutes after which the slide and the cover slip, still separated, were transfered into a tray containing xylene. This was then left for another 2 - 3 minutes to ensure the clearing of the cytological materials. Both slide and the cover slip were then removed from xylene and with the aid of the mark made on slide, the cover slip was mounted back appropriately, using Canada Basalm as mountant. The permanent slide was then adequately labelled and stored.

(iv) MEIOTIC STUDIES

Due to inavailability of flower buds of D. filamentosa during the time of this research, only the meiotic studies of D. longifolium

and D. tacazzeanum were carried out. Future work will adequately take care of the meiotic study in D. filamentosa.

Meoitic chromosomes were studied so as to confirm the chromosome numbers established by mitotic studies and to investigate the homology and pairing patterns of the chromosomes. These studies are useful as they throw more light on the possible origin of the chromosome complement and to detect the presence or otherwise of chromosomal changes such as deletions, translocations, inversions, or duplication of segments.

(a) **Choice of Materials**

Flower bud at suitable age contains anthers and ovules in which sporogenesis is in progress. With six anthers in a bud, each with numerous pollen mother cells in each of the two thecae, the anther was considered a better source of material, for meiotic chromosome study than the ovules which were difficult to reach and relatively very few in number.

The age at which spore formation was in progress was somehow difficult to determine. However, the size of the bud and inflorescence proved to be a useful guide. In addition, random trials of different stages of flower buds usually gave good result, since the meiotic activity depends on species and time of collection.

(b) **Collection of Materials**

For meiotic studies young flower buds that were about 2.5 - 3mm long were collected between 9.00 - 10.00a.m which provided the most suitable materials for the study. This is because during the day, the heat from the sun is known to cause clumping of the chromosomes. Buds of the right size were harvested using a pair of sterilized fine forceps. The perianth segments were opened out with a fine mounted needle to ensure the quick penetration of the fixative into the anthers and instant fixation. Harvested flower buds were then fixed immediately in freshly prepared 1:3 (V/V) acetic alcohol. The specimen tube was kept in the refigerator at 4°c for at least twenty-four hours before the materisl were further treated.

(c) **Preparation of Meiotic Materials**

The anthers for squashing were removed from the fixative, washed thoroughly in changes of distilled water to removed all traces of fixative arouna the anthers for easy hydrolysis. They were hydrolysed in five normal hydrochloric acid (NHcl) for about 8 - 10 minutes at normal - laboratory temparature (Oyewole, 1967). This was done to

soften the tissue by dissolving the middle lamella of the cells so that squashing will be possible. One anther at a time was removed and placed on a clean glass slide while the rest for squashing remain in distilled water. The anther was pressed in a drop of acetic orcein stain and the sporogenous tissue oezed out. The tapetal wall was then discarded. The preparation was then gently covered with a cover slip and gently passed over the flame of a spirit lamp. For proper spreading of the chromosome a little pressure was then applied by placing the slide face-down in between two folds of filter paper.

(d) **Investigation of Pollen Mother Cells**

Each meiotic preparation was exhaustively scanned under a light microscope at x40 objective. The behaviour of the chromosomes at the prophase stages, metaphase I and II, anaphase I and II and telophase I and II, were carefully studied. The prophase I stages studied were pachynema, Diplonema and Diakinesis.

These stages were studied to find out the pattern of pairing, whether any of the apparent forms were results of and or reunion of the non-sister chromatids. The metaphase

I chromosomes, being at their maximum contraction, were counted so as to confirm the chromosome numbers established earlier by mitotic studies. Metaphase II was examined to find out any unequal separation that might have occurred during the first anaphase.

Anaphase I and II were studied to find out whether any of the chromosomes might be forming laggards, non-disjunctions and or dicentric bridges. Early telophase I and II were investigated to check the probable "Left out" or excluded chromosomes outside the daugther nucles.

The late telophase I and II were also examined to investigate the presence of any micronuclei, number of daughter nuclei and their separation. As the shortening and thickening of the chromosomes during meiosis is a progressive process from leptonema to the end of full metaphase I, bivalent length was not measured. This was because a range of stages in division was present in any one anther and cells of exactly comparable conditions could not be identified with certainty. Note was also taken of cytokinesis I and II in the two species under investigation.

(e) **Photomicrography**

Intact cells with well spread chromosomes (or those which burst but whose contents were still intact) were photographed. Stages that were particularly photographed were pachynema, diplonema, diakinesis or metaphase I, Anaphase I and II, and telophase I and II. Photographic materials used and method employed were as detailed for the mitotic chromosome study.

A = D. tacazzeanum

B = D. filamentosa

C = D. longifolium

NOTE:

The centromeric value r is given as the ratio of long arm to short arm. L/S (Levan et al (1964). The r value was employed in determining the centromeric location according to the method of Levan et al (1964) as modified by Oyewole (1971).

KEY

C = Chromosme length

L = Long arm

S = Short arm

r = Centromeric value

Loc = Location of centromere

CHAPTER THREE

RESULTS

KARYOTYPE DATA OF THE THREE TAXA OF DIPCADI

The haploid number is represented in each case.

Table 1 **Measurements are in ùm**

Homologues		A	B	C
	c	12.20	10.0 *	7.10 *
1	r	10.09	8.09	10.86
	Loc	t	t	t
	c	10.90	8.90	7.0
2	r	9.8	6.80	22.30
	Loc	t	st	t
	c	9.30 *	7.0	6.60 *
3	r	7.45	6.0	12.20
	Loc	t	st	t
	c	7.0	5.80	5.90 *
4	r	6.0	7.2	22.60
	Loc	st	t	t
	c	5.3	4.75	5.60
5	r	3.0	3.52	10.40
	Loc	sm	st	t
	c	4.4	3.5	5.50 *
6	r	3.8	2.18	10.00
	Loc	st	sm	t
	c	-	-	5.10
7	r	-	-	11.75
	Loc	-	-	t
	c	-	-	4.10
8	r	-	-	9.25
	Loc	-	-	t
	c	-	-	3.05
9	r	-	-	19.33
	Loc	-	-	t
	c	-	-	2.75
10	r	-	-	2.26
	Loc	-	-	t
	c	-	-	2.70
11	r	-	-	10.0
	Loc	-	-	t
	c	-	-	2.25

PLATE 2

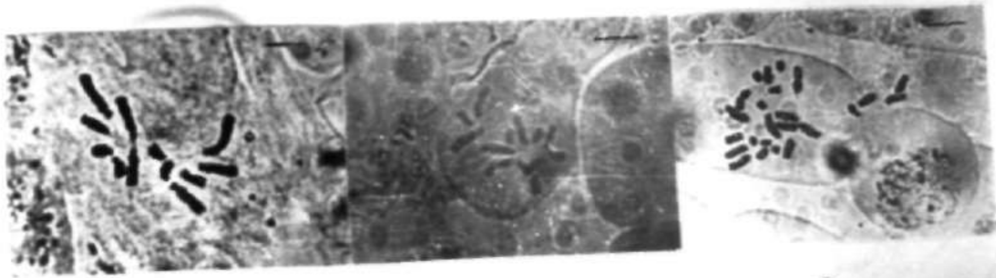
Photomicrographs showing metaphase somatic chromosomes of the three taxa of Dipcadi

A - D. tacazzeanum

B - D. filamentosa

C - D. longifolium

Bar represents 5um


A
B
C

12	-	-	-
Total Chromatin length =	98.20µm	79.90µm	115.3µm
$\bar{x}$	8.183	6.660	4.804

A = D. tacazzeanum

B = D. filamentosa

C = D. longifolium.

* Represent chromosome pair with secondary constriction.

SOMATIC CHROMOSOMES

Two different ploidy levels of chromosome complement were observed from the study. Dipcadi filamentosa and D. tacazzeanum were both observed to be diploid with 2n = 12, while Dipcadi longifolium was found to be 2n = 24, a tetraploid, confirming Oyewole (1988). Each complement consisted of a graded series of chromosomes with centromere position ranging from subterminal to terminal.

It is improtant to note that all the three groups studied possess secondary constrictions. Table 1 and 2 contains the summary of the karyotype data. plate 1 represents the morphological representation of the three taxa, while plate 2 consists of the photomicrographs of the metaphase spread of the somatic complements of D. tacazzeanum, D. filamentosa and D. longifolium respectively. Plate 3 represents the idiograms of the three taxa.

Dipcadi tacazzeanum

This possesses a somatic chromosome complement of 2n = 12 which are resolvable into six homologous pairs. Chromosome length ranges between 4.4um and 12.20um with total haploid chromatin length of 49.10um and the karyotype consists of three long and three short pairs. The fifth pair has a submedian centromere, while all the others have terminal to sub-terminal centromeres. The third pair has a secondary constriction inthe long arm.

Dipcadi filamentosa

The karyotype is represented by twelve chromosomes which can be resolved into six homologous pairs. Chromosome length ranges between 3.5um and 10.0um, with a total haploid chromatin length of 39.95um. Three pairs which are 8.90um, 7.0um and 4.75um long have their centromere at subterminal region, two pairs which are 10.00um and 5.8um long have their centromere located at the terminal region while the last pair with length 3.50um long has its centromere located at the submedian region. The first and the longest chromosome pair possesses a secondary constriction where one member's secondary constriction is wider than the second member.

D. longifolium

Dipcadi longifolium has a somatic complement of 2n = 24. The complement consists of sixteen long and eight short chromosomes, with

PLATE 3

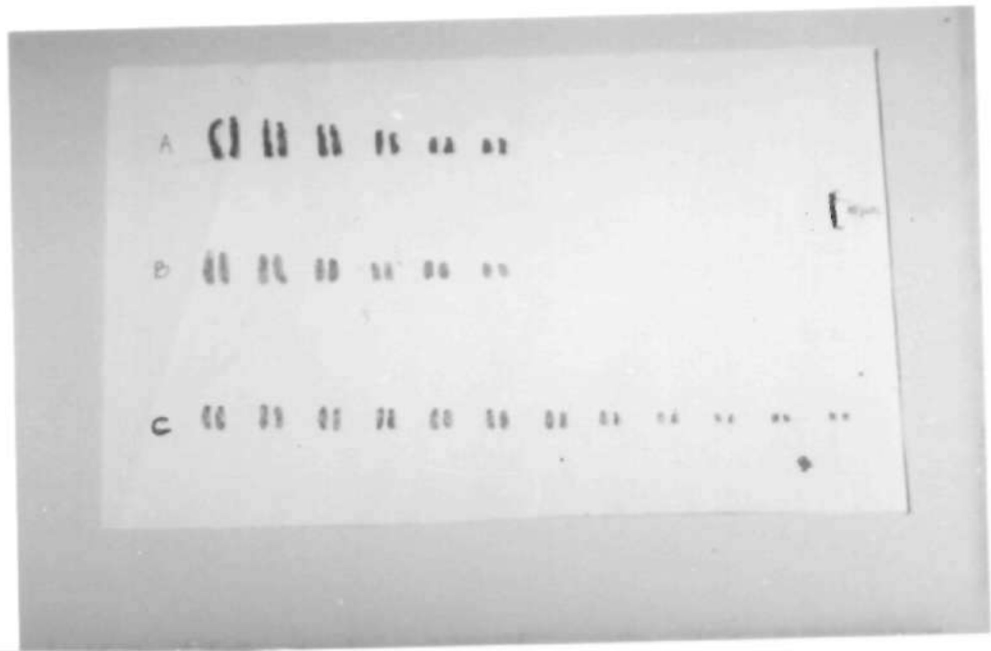
Idiograms of the three taxa of Dipcadi

A - D. tacazzeanum

B - D. filamentosa

C - D. longifolium

Bar represents 10um


A
B
C

chromosome lengths ranging from 2.2um to 7.10um. One pair of short chromosomes is telocentric, another has submedian centromere, while the remaining two short and all the eight long pairs have terminal to sub terminal centromeres. One of the short pairs with the centromere in the terminal region varies morphologically in different individuals - one or both members have an extended centromeric region. Four pairs (1st, 3rd, 4th and 6th) have a secondary constriction each in the long arm.

CENTROMERIC LOCATION OF THE THREE TAXA OF DIPCADI

Table 2:

	T	t	st	sm	m	M
A	-	3	2	1	-	-
B	-	2	3	1	-	-
C	1	10	-	1	-	-

A = D. tacazzeanum

B = D. filamentosa

C = D. longifolium

NOTE:

The centromeric value r is given as the ratio of long arm to short arm, L/S (Levan et al, 1964). The r-value was employed in determining the centromeric location according to the method of Levan et al (1964) as modified by Oyewole (1971).

KEY

C = Chromosome length

L = Long arm

S = Short arm

r = Centromeric value

Loc = Location of centromere

MEOITIC CHROMOSOMES (SEE TABLES 3 & 4)

The various stages of meiotic cell division observed showed that chromosome behaviour was normal in both D. tacazzeanum and D. longifolium. Univalents, laggards or eliminated chromosomes and multivalents were of rare occurrence in any of the two taxa. Tetrad of spore formation was quite normal and regular in both taxa.

D. tacazzeanum

Six bivalents were observed at the pachytene stage. The pollen mother cell was columnar in shape and average chiasma frequency was twelve per complement. At anaphase I and II, chromosome movement was normal. Mature microspores were spherical in shape.

D. longifolium

Pachytene stage showed that there were twelve bivalents. The pollen mother cell was columnar in shape. Average chiasma frequency per complement was found to be twenty four. Anaphase I and II chromosome movement was normal. Cytokinesis I followed TI. Meiotic products were normal. Tetrad arrangement was linear or decussate. The four mature spherical microspores were all of equal size.

PLATE 4

Photomicrographs showing meiosis in D. longifolium

A - Pachytene stage in D. longifolium.

B - Diplotene stage in D. longifolium.

C - Metaphase stage in D. longifolium.

D - Clean separation at anaphase I

E - Early Telophase I in D. longifolium.

F - Diad formation in D. longifolium.

G - Anaphase II (Early separation).

H - Anaphase II (Clean separation).

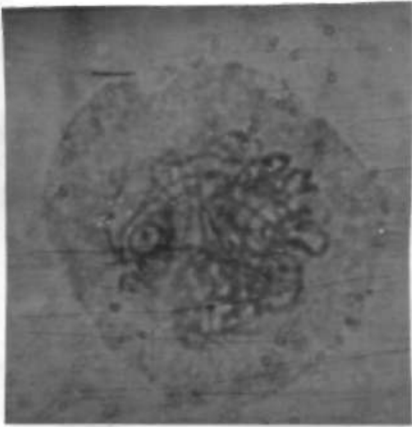
I - Anaphase II (With extension of cytoplasm).

J - Tetrad of spore.

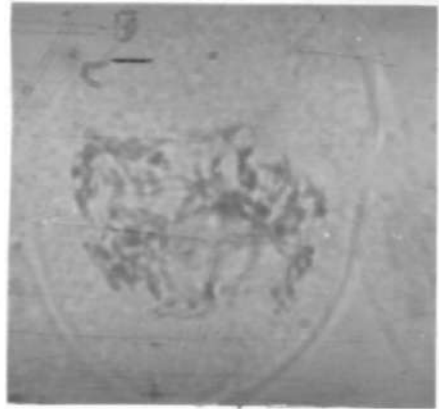
L - " " " (linear).

M - Pollen grain.

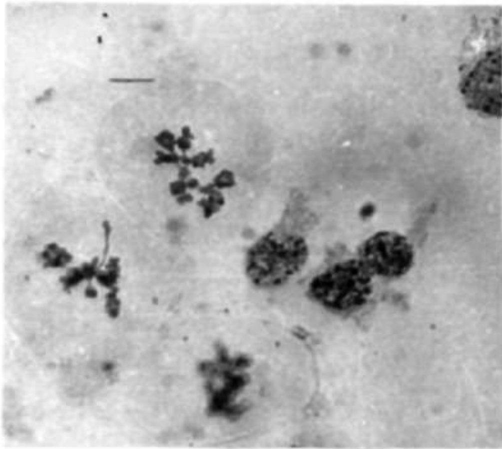
— Represents 5μm.



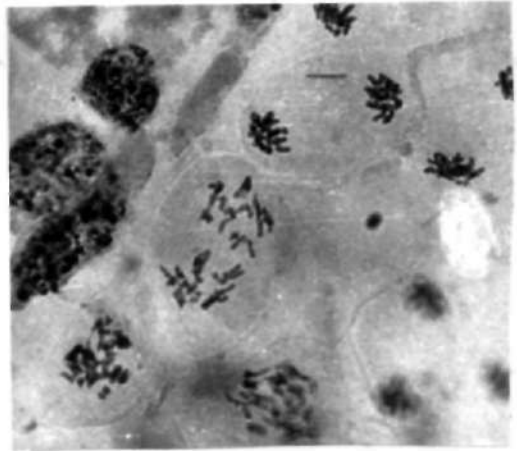
A



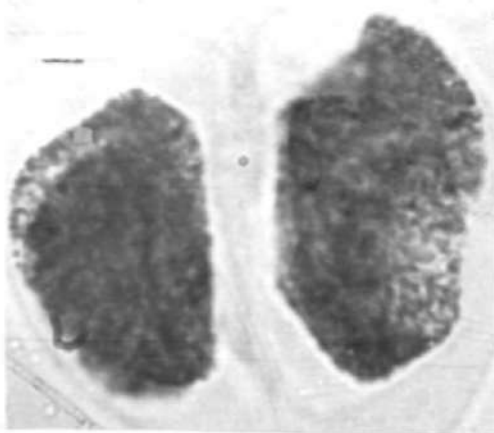
B

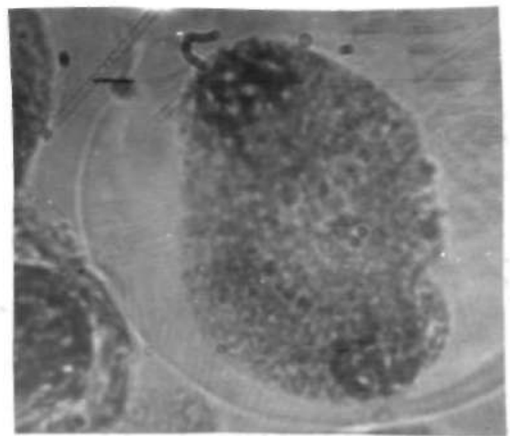


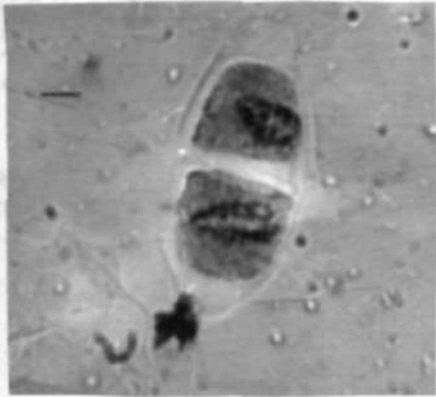
C



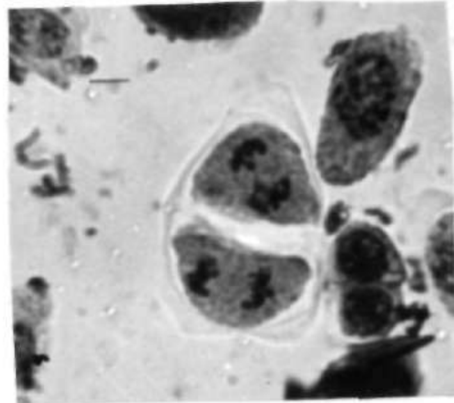
D



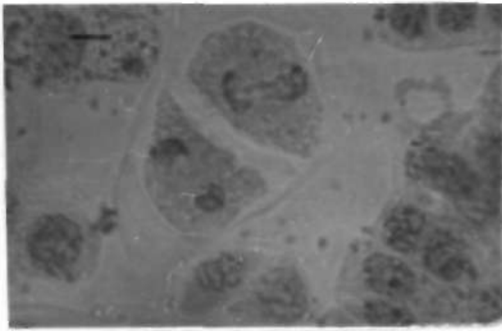




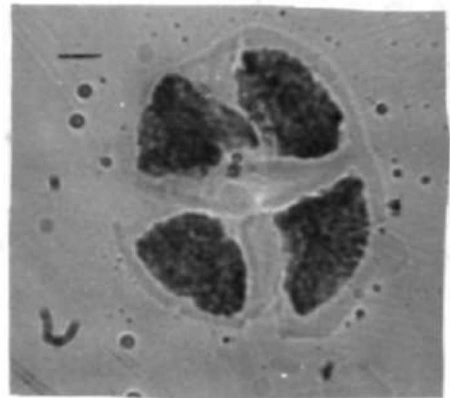
G



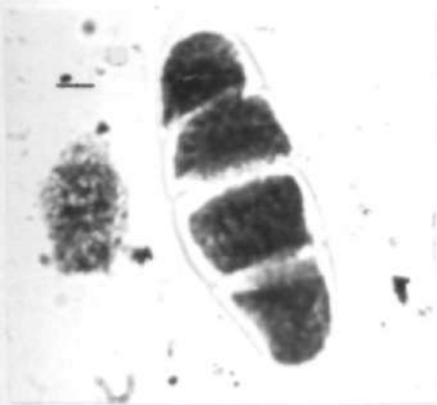
H



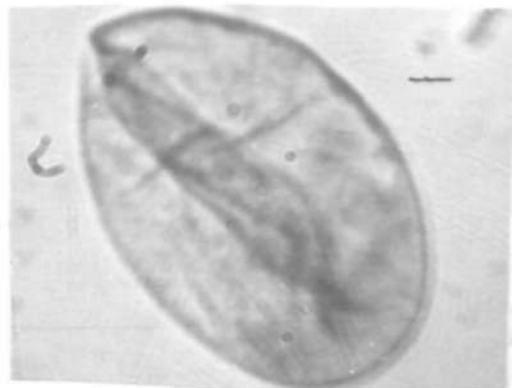
I



J



L



M

PLATE 5

Photomicrographs showing meisosis in D. tacazzeanum

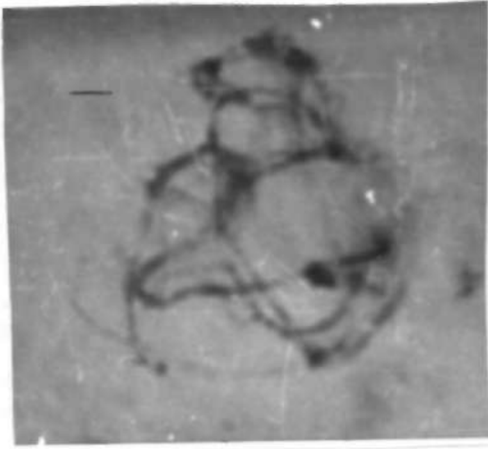
i. - Pachytene stage

ii. - Metaphase stage

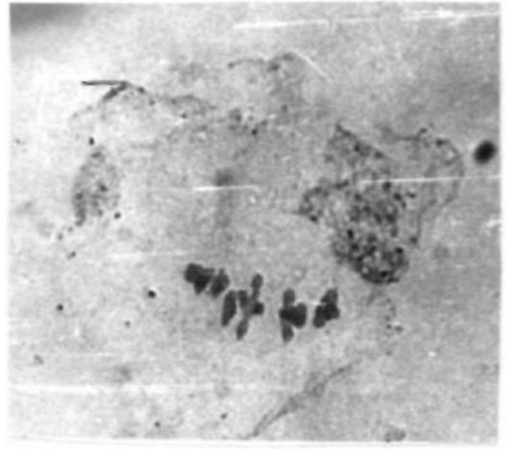
iii. - Early anaphase I showing clean separation

iv. - Telophase I and II

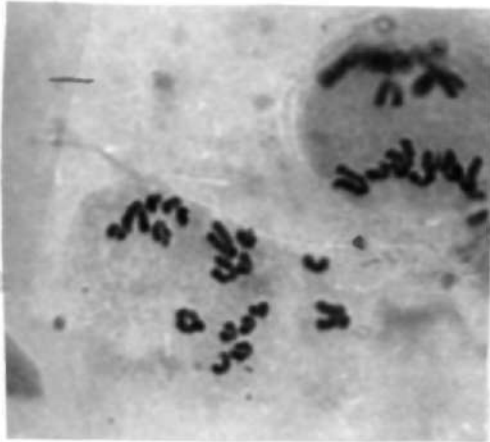
—— Represents 5um.



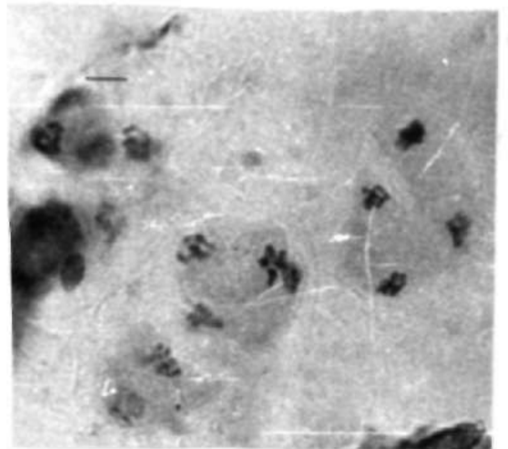
i



ii



iii



iv

Table 3:

BIVALENT	A	C
1	2	2
2	2	2
3	2	2
4	2	2
5	2	2
6	2	2
7	-	2
8	-	2
9	-	2
10	-	2
11	-	2
12	-	2
$\bar{X}$	2.0	2.0

A = D. tacazzeanum

B = D. longifolium

Table 4: **SUMMARY OF MEIOTIC CHROMOSOME BEHAVIOUR IN D. tacazzeanum and D. longifolium**

CHARACTERS	A	C
Pollen mother cell	Columnar	Columnar
Pairing of chromosomes	Regular	Regular
Number of bivalents per complement	Six	Twelve
Chiasma frequency/ complement at diplotene	Twelve	Twenty four
Anaphase I	Normal	Normal
Anaphase II	Normal	Normal
Anaphase Bridge	Absent	Absent
Cytokinesis I	Present	Present
Cytokinesis II	Present	Present
Diad nuclei	Uniform	Uniform
Tetrad nuclei	Equal	Equal
Microspore shape	Round	Round
Microspore size	Not equal	equal

A = D. tacazzeanum

B = D. filamentosa

CHAPTER FOUR

DISCUSSION

One of the most important influences on taxonomy during the past few decades has been the contribution of cytology either alone (cytotaxonomy) or interwoven with genetics (cytogenetics). Cytological features such as chromosome numbers and morphoiogy can be used like any other kind of comparative data. Chromosme behaviour and structure at meiosis contribute to our understanding of the evolution and relationships of population. Cytological results have been found useful in solving taxonomic problems. This is because chromosome is a stable and definite species character. Chromosome studies have been used by workers such as Bocher et al (1953) and Focher (1959) to correlate differences in chromosome number or morphology with morphological differentication or ecological distribution or both. Knowledge of chromosome shape and size in related taxa can be of fundamental importance to evolutionist and taxonomist alike. Jones and Smith (1967) contended that the study of karyotype is particularly rewarding in such families as liliaceae where large chromosomes and frequent bimodality in size within the complements make possible the determination of the progress of chromosome changes and its consequences.

Cytological investigation of Nigerian representatives of Dipcadi so

far conducted and reported is very meagre. Jones and Smith (1967) reported a somatic chromosome number of 2n = 12 for a diploid species suspected to be D. gracillium. The records of 2n = 8, 18 and 34 for three different species, by which the basic numbers of X = 4,9 were determined, were from Southern African materials (see Darlington & Wylie, 1955). Investigation on Chromosome counts and karyomorphology of some West African Scilleae indicated that the basic chromosome number of the Nigerian representatives of the genus is X = 6, Oyewole (1988). This number was also confirmed in this study. Two ploidy levels were also confirmed inthe genus, D. tacazzeanum and D. filamentosa were found to be diploid with chomosome number of 2n = 12 while D. longifolium was confirmed to be a tetraploid with somatic chromosome number of 2n = 24. These findings also agree with the chromosme numbers obtained from chromosme count of some West African Scilleae by Oyewole (1988). The organisation of the centromere at the sub terminal and terminal position of the chromosomes which is also typical of the family liliaceae was also confirmed in the three taxa investigated. However, all of them have similar basic plan whereby each complement consisted of a graded series of chromosomes with centromere positon ranging from sub terminal to terminal.

In karyotype differentaition, the existence of two ploidy levels indicates:

(i) the separation of D. longifolium from others and

(ii) the recent geneology for the genus in West Africa.

The distinctiveness of the karyotype of D. tacazzeanum and D. filamentosa establishes the specific differentiation between the two taxa and therefore invalidates the inclusion of D. filamentosa in D. tacazzeanum by Hepper (1968).

The chromosome length bimodality in certain members of the tribe Scilleae has been reported (Jones & Smith, 1967; Oyewole, 1972, 1975b). This has held true in the present work for D. tacazzeanum and D. filamentosa, while lack of bimodality in D. longifolium indicates that its chromosome complement has suffered changes of the type of loss of minute segments without leaving any remarkable identifiable landmarks in the meiotic behaviour. The presence of morphologically unidentical members of the same pairs of chromosomes in each of the three taxa - unequalness in length, unequalness of centromeric region or secondary constriction area is an evidence of hybridity of origin of the three taxa. The differences in karyomorphology of the different taxa seem minute, but they are basic and do underlie the differences in the external morphology of each taxon. It is therefore, evident that changes have occurred or are occurring in these taxa that may be correlated with morphological differentiation of the populations as rightly pointed out by Oyewole (personal communication). The differences

in the amount of chromatin materials may have resulted from or led to karyotype differentiation. The results of this study as shown by the similarity in some sets of chromosome among the three taxa indicates that:

(a) D. tacazzeanum and D. filamentosa show close karyomorphology and similar sets of chromosome could be figured out between both of them e.g the telocentric chromosomes with length 10.90 m and 10.0 m, subtelocentic chromosmes with length of 7.0 m in both and the subtelocentric chromosomes with length of 4.4 m and 4.75 m. With this genomic closeness, it is therefore possible that both of them have common progenitors.

(b) While the diploids are related, they do not represent the same taxon.

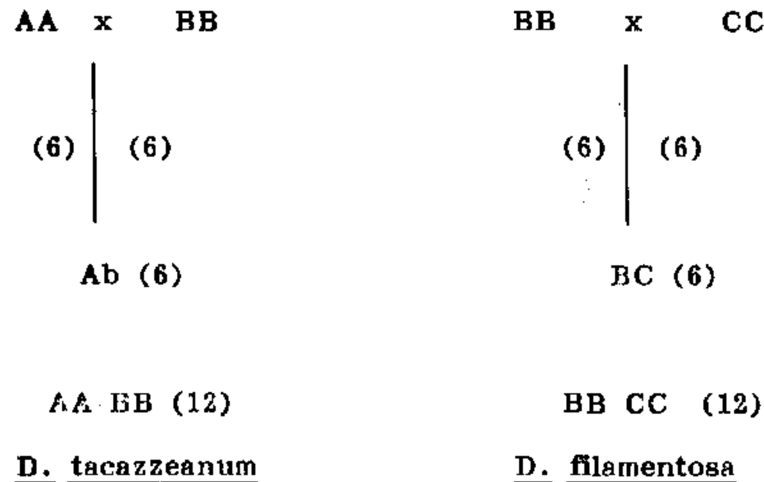
(c) A cross between this same progenitor and their ancestral parent followed by chromosome doubling could have led to D. longifolium.

(d) The three taxa are of common ancestry.

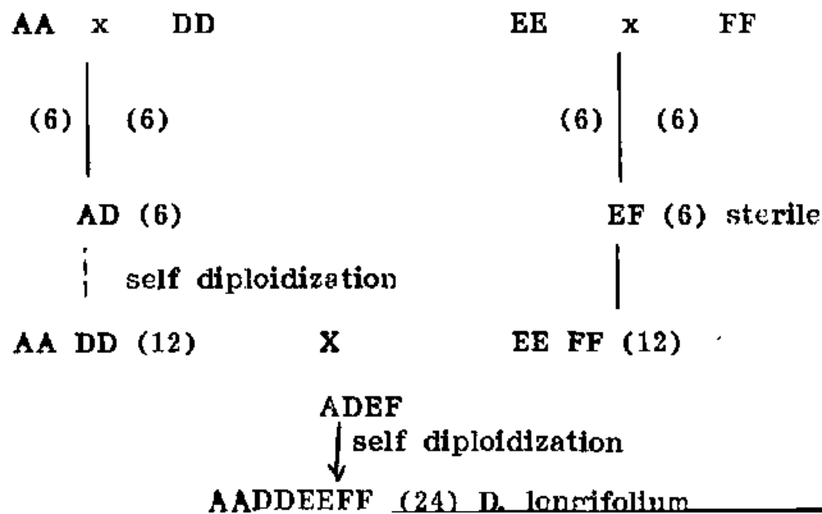
(e) The three taxa are all of hybrid origin.

(f) The occurence of sets of three homologous chromosome pairs give the impression that the basic chromosome number of the ancestral stock of Dipcadi is most likely to be X = 3, from which the present taxa evolved. From these deductions, therefore, a

possible and reasonable mode of evolution of Nigerian *Dipcadi* could be postulated. If the ancestral parents are 2x = 6, then *D. tacazzeanum* and *D. filamentosa* would have had one common parent as follow:



The set of three homologous chromosome pairs which are similar in both *D. tacazzeanum* and *D. filamentosa* is the BB. The BB would be the common progenitor in *D. tacazzeanum* and *D. filamentosa*. Hence, the probable mode of evolution of *D. longifolium* will be as stated below:



Speciation in plant involve the formation of such natural alloploids, through hybridization and diploidization (Stebbins 1974; Sears 1941; Sears and Okamoto, 1958; Oyewole 1972). The loss of identicality in those sets of chromosomes that look alike in pair of taxa and which are presumed to have originated in the same way might have involved aberrations of chromosomes resulting from adaptation to new ecological niches. Such aberrations are likely to involve segmental loss of homologous chromosomes.

Meiotic behaviour, which is normal in both D. tacazzeanum and D. longifolium, indicates that the genetic systems of both taxa are stable. The absence of univalents and multivalent formations indicates that both taxa are functional diploids. Hence, D. longifolium, a tetraploid is not autotetraploid. It must have arisen by natural hybridization between related diploids, followed by autodiploidization to produce the functional diploid (amphidiploid). It is therefore an allotetraploid.

The regularity of meiosis in both D. tacazzeanum and D. longifolium confirms a basic number of X = 6 (Oyewole, 1988) for West African awned squills. The regular distribution of chromosomes and absence of laggards ensure balanced gametes, with each pollen grain recieving a complete haploid chromosome complement. Swanson (1968) reported that the uniformity in size of intraspecific pollen grains is due to the regular distribution of chromosomes during meiosis. There is marked uniformity in the size of intraspecific pollen grains in this investigation.

CONCLUSIONS

Cytological investigation shows that _D. tacazzeanum_ and _D. filamentosa_ have twelve chromosomes per complement (diploid), while _D. longifolium_ has twenty four chromosomes per complement (tetraploid). They were all found to be of hybrid origin. Though the two diploids have the same number of chromosomes per complement, the differences shown in their chromosome sizes and total chromatin length definitely provide a strong indication for putting them in separate species. The chromosome length bimodality in _D. tacazzeanum_ and _D. filamentosa_ is in line with the findings in other members of the tribe Scilleae while lack of bimodality in _D. longifolium_ indicates that its chromosome complement has suffered changes of type of loss of segments without leaving any identifiable landmarks in the meiotic behaviour.

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